FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
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U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	09/980134

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/15161	**1 June 2000**	**1 June 1999**

TITLE OF INVENTION

SURFACE STABILIZED MICROBUBBLES FOR USE IN ULTRASOUND CONTRAST AND DRUG DELIVERY AGENTS

APPLICANT(S) FOR DO/EO/US

BASUDE, Raghuveer and WHEATLEY, Margaret

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

    1) Courtesy copy of the International Application;
    2) Courtesy copy of Response to Written Opinion; and
    3) Return post card

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
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24. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1040.00**
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$890.00**
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$740.00**
☒	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$710.00**
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$710.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	14 - 20 =	0	x $18.00	**$0.00**
Independent claims	2 - 3 =	0	x $84.00	**$0.00**
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**
	TOTAL OF ABOVE CALCULATIONS =			**$710.00**
☒ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$355.00**
	SUBTOTAL =			**$355.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**
	TOTAL NATIONAL FEE =			**$355.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**
	TOTAL FEES ENCLOSED =			**$355.00**
				Amount to be: refunded $
				charged $

a. ☐ A check in the amount of _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **50-1619** in the amount of **$355.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **50-1619** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Jane Massey Licata, Registration No. 32,257
Kathleen A. Tyrrell, Registration No. 38,350
Licata & Tyrrell P.C.
66 East Main Street
Marlton, New Jersey 08053

Tel: 856-810-1515
Fax: 856-810-1454

Jane Massey Licata
SIGNATURE

JANE MASSEY LICATA
NAME

32,257
REGISTRATION NUMBER

November 29, 2001
DATE

DRE-0063

CERTIFICATE OF EXPRESS MAILING

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I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R 1.10 on the date indicated above and is addressed to the "BOX PCT", U.S. Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202.

1) Transmittal Letter (2 copies) with authorization to charge deposit account $355.00 for filing fee;

2) PCT International Application;

3) International Search Report;

4) International Preliminary Examination Report;

5) Response to Written Opinion; and

6) Return Postcard;

Jane Massey Licata
JANE MASSEY LICATA

Surface Stabilized Microbubbles for Use in Ultrasound Contrast and Drug Delivery Agents

This invention was supported in part by funds from the U.S. government (NIH Grant No. HL 52901 and CA 52823) and the U.S. government may therefore have certain rights in the invention.

Background of the Invention

Ultrasound contrast agents are used routinely in medical diagnostic, as well as industrial, ultrasound. For medical diagnostic purposes, contrast agents are usually gas bubbles, which derive their contrast properties from the large acoustic impedance mismatch between blood and the gas contained therein. Important parameters for the contrast agent include particle size, imaging frequency, density, compressibility, particle behavior (surface tension, internal pressure, bubble-like qualities), and biodistribution and tolerance.

Gas filled particles are by far the best reflectors. Various bubble-based suspensions with diameters in the 1 to 15 micron range have been developed for use as ultrasound contrast agents. Bubbles of these dimensions have resonance frequencies in the diagnostic ultrasonic range, thus improving their backscatter enhancement capabilities. Sonication has been found to be a reliable and reproducible technique for preparing standardized echo contrast agent solutions containing uniformly small microbubbles. Bubbles generated via this technique typically range in size from 1 to 15 microns in diameter with a mean bubble diameter of 6 microns (Keller et al. *J. Ultrasound Med.* 1986 5(9):493-8). However, the durability of these bubbles in the blood stream has been found to be limited, providing impetus for a number of approaches to further stabilize them.

The half-life of free microbubble solutions has been reported to range from 44 ± 12 seconds for Hypaque 50%, to 253 ± 73 seconds for Iopamidol. Addition of a surfactant to dextrose 70 wt% has been reported to prolong bubble half life from 58 ± 12 seconds to 1018 ± 276 seconds (Keller et al. *J. Ultrasound Med.* 1986 5(9):493-8).

Surfactant stabilized microbubble mixtures for use as ultrasound contrast agents are also disclosed in U.S. Patent 5,352,436.

WO 9847540 discloses a contrast agent for diagnostic ultrasound and targeted disease imaging and drug delivery comprising a dispersion of a biocompatible azeotropic mixture, which contains a halocarbon.

WO 9729783 discloses a material useful as a contrast agent which comprises an aqueous dispersion of gas microbubbles stabilized by amphiphilic material containing phospholipid molecules having an overall net charge.

U.S. Patent 5,695,740, U.S. Patent 5,567,415 and U.S. Patent 5,701,899 disclose a pharmaceutically acceptable ultrasound contrast agent comprising microbubbles with an internal atmosphere enhanced with a perfluorocarbon gas.

WO 9421301 discloses an ultrasound agent consisting of a biocompatible oil-in-water emulsion in which the oil phase comprises an oil-soluble gas/fluid or gas precursor.

U.S. Patent 5,637,289, U.S. Patent 5,648,062, U.S. Patent 5,827,502; and U.S. Patent 5,614,169 disclose contrast agents comprising water-soluble, microbubble generating carbohydrate microparticles, admixed with at least 20% of a non-surface active, less water-soluble material, a surfactant or an amphiphillic organic acid. The agent is prepared by dry mixing, or by mixing solutions of components followed by evaporation and micronizing.

U.S. Patent 5,686,060 describes an injectable suspension for ultrasonic echography comprising a carrier liquid containing at least 10^7 microbubbles per milliliter and at

least one saturated phospholipid at a concentration below 0.01% by weight. Also disclosed is a method of producing the suspension of air or gas filled microbubbles which comprises dissolving a surfactant and stabilizer in an organic solvent; freeze drying the solution to form a dry powder; contacting the powder with air or another gas; and admixing the powder with the aqueous carrier.

EP699445 describes a method of preparing a stable microbubble solution for use as an imaging agent via a surfactant mixture containing a sodium salt of saturated carboxylic acids and saponin, stearic acid, phloxine, crystal violet, polyvinyl alcohol and/or sodium laurate. In this method, bubbles are formed in an aqueous solution by mixing with a machine having a 0 to 20000 rpm shaft and simultaneously introducing gas. The dispersion of microbubbles produced is poured into a stopcock bottomed tube and left to stand. The microbubble solution is then collected from the tube bottom and the surfactant mixture is added to change the nature of the bubble surface.

Stabilized sulfur hexafluoride (SF_6) microbubbles, referred to as BR1, have also been evaluated for use as an echo contrast agent (Schneider et al. *Invest. Radiol.* 1995 30(8):451-7). BR1 is formulated as a lyophilized product, which after addition of saline, provides a suspension containing 2 x 10^8 SF_6 microbubbles/ml with a mean diameter of 2.5 microns. The use of SF_6 rather than air provides improved resistance to pressure increases such as those occurring in the left heart during systole. After reconstitution, the echogenicity and bubble characteristics remain almost constant for 8 hours. BR1 injections in animals resulted in a homogenous, dose-dependent opacification of the left heart. Accordingly, BR1 is suggested to be a promising ultrasound contrast agent.

Micrometer-sized porous particles or "nanosponges" with properties suitable for entrapment and stabilization of the gas

bubbles due to an irregular complex surface morphology have also been developed. The complex morphology and surface chemistry involved in the production of these nanosponges makes it unfeasible to directly measure the volume of entrained gas (Phillips et al. *Ultrasonics* 1998 36(8):883-892). Accordingly, a model based on acoustic scattering principles has been proposed which indicates that only a small volume fraction of the gas should be necessary to significantly enhance the echogenicity of this type of particle-based contrast agent. In this model, the effective scattering cross-section is evaluated as a function of the volume fraction of gas contained in the overall scatterer and the overall scatterer diameter. Initially, the volume fraction of gas is considered as a discrete entity of single bubble. Using common mixture rules, it is then shown that the gas can be considered to be distributed throughout the particle and still arrive at a result that is similar to that for a single, discrete volume of gas. The main contribution to the increased scattering cross-section is due to the compressibility difference between gas and water. The backscatter coefficient is computed as the product of the resulting differential scattering cross-section and the scatterer number density. Clinical use of these nanosponges is suggested.

Encapsulated microbubbles typically last longer than free bubbles. In addition, encapsulated microbubbles can also be used as drug delivery agents. For example, microvessel rupture caused by insonification of thin polymer-shelled microbubbles *in vivo* has been suggested as a minimally invasive means for delivering colloidal particles and engineered red blood cells across the endothelial lining of a targeted tissue region (Price et al. *Circulation* 1998 98(13):1264-7). However, for ultrasound procedures encapsulation of bubbles can alter their scattering properties thereby lowering enhancement as compared to free bubbles.

In the present invention, a technique is provided for combining the benefits of both free bubbles and particles into surface stabilized microbubbles, the surface being able to take the form of a microparticle, or a surface coating of an object such as a biopsy needle or radioactive seed.

Summary of the Invention

An object of the present invention is to provide surface stabilized microbubbles. In one embodiment, the surface stabilized microbubble is produced by introducing microparticles having hydrophobic surface properties and which have been stored in a gaseous environment into a liquid so that the microparticle carries with it some gas into the liquid, thereby creating a microbubble attached to or encapsulating the microparticle.

In another embodiment, the surface stabilized microbubble is produced by introducing microparticles with an affinity toward a specific gas and which have been stored in that gas into the liquid.

In yet another embodiment, surface stabilized microbubbles are produced by insertion of a hydrophobic surface into a medium which contains a relatively hydrophobic dissolved gas such as oxygen, or nitrogen, which spontaneously comes out of solution and forms on the hydrophobic surface. In yet another embodiment gas bubbles that are present or generated in the solution attach themselves to the introduced hydrophobic surface. In this embodiment, gas bubbles may be generated by a variety of methods including, but not limited to, due to agitation, homogenization, sonication, decompression, phase shift, or chemical effervescence. Surface stabilized microbubbles of the present invention are useful as ultrasound contrast and drug delivery agents and to create ecogenic surfaces on objects to enhance ultrasonic detection of the object.

Brief Description of the Drawings

Figure 1 shows a schematic of the *in vitro* apparatus used to measure the acoustic properties of the surface stabilized microparticles of the present invention.

Detailed Description of the Invention

In the present invention, a surface stabilized microbubble technique is provided to produce ultrasound contrast agents and ecogenic surfaces which enhance ultrasound detection of objects. This surface stabilized microbubble technique is also useful in development of novel drug delivery systems. In one embodiment, the technique of the present invention utilizes microparticles having hydrophobic surface properties or with an affinity toward a specific gas. When a dry, relatively hydrophobic microparticle from a gaseous environment is introduced into a liquid such as buffer, water or blood, the particle carries with it some of the gas into the liquid, thereby creating a microbubble which attaches to or encapsulates the microparticle. Similarly, when a hydrophobic microparticle is introduced into a solution containing a dissolved gas, tiny gas bubbles can spontaneously form on the surface of the microparticle from the solution. Based upon surface characteristics of the microparticles such as shape, degree of hydrophobicity relative to the suspending fluid and actual area that is hydrophobic, the gas bubble may wholly encapsulate the particle or attach/adhere itself to part of the particle. Alternatively, microparticles can be used which have an affinity for a specific gas. In this embodiment, surface stabilized microbubbles can be created by storing microparticles with an affinity toward a specific gas in the specific gas and then introducing the microparticle into a liquid so that the microparticle carries with it some gas in which it was stored into the liquid so that a gas microbubble attaches to or encapsulates the microparticle.

Microparticles useful in the present invention may be solid or hollow and may comprise organic or inorganic compounds and even living components. These can include solid or hollow microparticles or surfaces of non-biodegradable polymers such as teflon, poly vinyl alcohol, polystyrene and polyethylene and biodegradable polymers such as poly anhydrides, poly esters, starch, cellulose, and ethyl cellulose. The particles may include encapsulated or adhered drugs or cells such as genetically engineered cell lines which can excrete specific desired factors such as growth or necrosis factors. Microparticles may be spherical or irregular in shape. However, microparticles or surface coatings used in the present invention must be partially or completely coated or made up of, at least in part, a relatively hydrophobic component. Alternatively, the microparticles or surface coatings must have an affinity for a selected gas.

The microbubble portion of the surface stabilized microbubble can be formed by any gas. Examples include, but are not limited to air, SF_6, noble gases such as xenon, and PFCs.

Further, a targeting moiety such as an antibody can also be attached to the surface stabilized particle.

As demonstrated herein, surface stabilized microbubbles have backscattering characteristics which render them useful in ultrasound contrast.

Surface stabilized microbubbles of the present invention were tested using equal weights (0.5 grams) of microparticles with varying hydrophobicities. Specifically backscattering enhancement as a function of time was determined individually for starch (Sigma, Missouri, USA), talc (Baby powder, CVS ,USA or Plastodent Inc. NY USA) and polyethylene (Shamrock Technologies Inc. NJ, USA) microparticles alone or in the presence of a surfactant. The level of backscattering enhancement was consistent with the extent of the particle's surface hydrophobicity. Polyethylene and talc showed excellent

backscattered enhancement (>30 dB). The surface stabilized microbubbles in both cases were stable at the same level of enhancement over a period of 15 minutes. The enhancement of polyethylene at a dose of 0.5 grams was masked by shadowing. Hence, additional tests were carried out at lower doses of 0.05 grams and 0.02 grams.

As will be obvious to those of skill in the art upon this disclosure, the techniques of creating surface stabilized microbubbles from microparticles are also applicable to larger surfaces of objects such as radioactive seeds or biopsy needles. Using these techniques, ecogenic surfaces can be created to enhance the ultrasonic detection of the object.

The preferred method of *in vivo* administration of surface stabilized microparticulate agents is via suspension of the lyophilized particulate surface in a physiologically acceptable buffer, followed by intravenous injection just prior to conducting an ultrasound scan. The microparticulates can be stored under an atmosphere of the desired gas, for example SF_6 or a PFC. In embodiments where an ecogenic surface is to be used, such as in an ultrasonically guided biopsy needle, the needle can be coated with a hydrophobic surface, and stored sterile either under vacuum or in the presence of a gas of choice. The vacuum stored object would be used under conditions where gas is expected to spontaneously form small microbubbles on the surface *in situ*. In embodiments wherein radioactive seeds are coated, for example in the prostate, the seeds would be pre-coated with the hydrophobic surface and stored sterile either in vacuum or in the presence of a gas of choice.

Surface stabilized microbubbles are also useful in drug delivery and targeting techniques. Since the microbubbles are stabilized at the surface of a polymer or particle, this polymer/particle can comprise a matrix containing a drug by incorporation or by surface binding, or can comprise drug particle itself. Surface stabilized microbubbles comprising

the drug can then be delivered to an imaged site by insonation of the surface/particle, causing the matrix to vibrate and release drug. It is also possible that the insonation will cause the particle to rupture, releasing part or all of any contents trapped within the matrix or within the hollow interior of the particle.

The following nonlimiting example is provided to further illustrate the present invention.

Example

Acoustic properties of the surface stabilized microparticles were measured in an in-vitro setup illustrated in Figure 1. A custom-built acrylic tank , (1),(30.5 x 26.7 x 25.4 cm) was filled with freshly degassed de-Ionized (~18 MΩ) water. An acrylic sample container (5 x 10 x 17.8 cm), having a 5 x 5 cm acoustic window (2) was filled with 750 ml of Phosphate buffer saline (PBS) {NaCl [8.01 grams], KCl [0.194 grams], Na_2HPO_4 [0.909 grams], and KH_2PO_4 [0.191 grams] in one liter of water}, and placed inside the tank at approximately 30 mm from the back of the tank and 75 mm from the sides. The cover of the tank was fitted with a x-y positioning system (Edmund Scientific, Barrington, NJ, USA) to mount the ultrasonic transducers. The contents of the sample container were constantly stirred using a magnetic stirrer (3). A single element, broadband, 12.7 mm (0.5") element diameter, 50.8 mm (2") point focussed transducer (Panametrics, Waltham, MA) with center frequency of 5 MHZ (4), was chosen to represent the conventional diagnostic ultrasound range. The -6 dB bandwidths of the transducer was 91.74%. A Panametrics 5072 PR pulser/receiver was used to drive the transducer in pulse-echo mode. The received signals from the transducer were fed to a digital oscilloscope, (5),(LeCroy 9350A, LeCroy Corporation, NY, USA). The digitized data from the oscilloscope were then stored and processed using Labview 4.1 (National Instruments, Austin, TX, USA) and a computer, (6), (PowerMac 7500/132).

The Panameteric 5052 (Panametrics, Waltham, MA) pulser/receiver was set as follows:

Rep. Rate = 100 Hz
Energy = 1
Damping = 3 (50 Ohms)
Gain (dB) = 10

"1-2" switch on position "1", this is the pulse-echo mode.

The transducer was aligned using the X and Y axis controls to obtain maximum amplitude of the signal. The transducer was then advanced towards the sample container by approximately 3 mm. Thus, the focus of the transducer lay 3 mm inside the sample container, which was approximately 7.5 μsec from the front wall echo. The gain of the amplifier was changed to 40 dB.

Gain (dB) = 40

Fifty readings (rms) of the of 2 μsec (7.5-9.5 μsec from front wall) time gated signal were taken at the focus without any surface stabilized microparticles. The average of these 50 readings was considered the reference level. Next, a known dose of surface stabilized microparticles, typically 0.5 grams/750 ml of PBS buffer, was administered inside the sample container. After a 10 sec delay, the average of 50 readings (rms) of the 2 μsec time gated signal at the focus was recorded. The enhancement due to the presence of contrast agent was determined as follows:

$$\sum_E = 20 \log_{10} [\mathrm{rms}\{s_{CA}(t)\}/\mathrm{rms}\{s_0(t)\}]$$

where,

$\sum_E$ = Backscattered Enhancement
$s_{CA}(t)$ = Average of 50 reading with contrast agent
$s_0(t)$ = Average of 50 readings without contrast agent (reference level).

The backscattered enhancement was recorded at every minute for 15 minutes, post administration of the agent. The results were plotted as backscattered enhancement (dB) versus time in minutes.

Three agents, namely, starch (Sigma), talc (Baby powder, CVS, USA or Plastodent Inc. NY, USA) and polyethylene microparticles (Shamrock Technologies Inc. USA) were chosen. Starch was the least hydrophobic, while polyethylene was the most hydrophobic of the three. A sample (0.5 grams) of the agent to be tested was taken and the backscattered enhancement with time was recorded. The measured amount of backscattered enhancement was consistent with the hydrophobicity of the test sample.

A second, similar set of experiments was conducted wherein the microparticles of either starch, talc or polethylene were coated with the surfactant, TWEEN 80 (monoleate polyoxyethylenesorbitan; Sigma Chemical Co., St. Louis, MO) to nullify the effects of surface hydrophobicity of the microparticles. This lead to dramatic decreases in enhancement in the case of polyethylene, and to a lesser amount in talc and starch, consistent with the degree of hydrophobicity that was nullified respectively.

What is Claimed is:

1. A surface stabilized microbubble comprising a microparticle having a hydrophobic surface or an affinity for a specific gas and a gas microbubble which attaches to or encapsulates the microparticle.

2. The surface stabilized microbubble of claim 1 produced by a method comprising:

(a) storing the microparticle in a gaseous environment; and

(b) introducing the microparticle into a liquid so that the microparticle carries with it some gas in which it was stored into the liquid so that a gas microbubble attaches to or encapsulates the microparticle.

3. The surface stabilized microbubble of claim 1 produced by a method comprising:

(a) storing the microparticle with an affinity toward a specific gas in the specific gas; and

(b) introducing the microparticle into a liquid so that the microparticle carries with it some gas in which it was stored into the liquid so that a gas microbubble attaches to or encapsulates the microparticle.

4. The surface stabilized microbubble of claim 1 produced by a method comprising introducing the microparticle having a hydrophobic surface into a liquid which contains a dissolved gas thereby creating a surface for the dissolved gas to come out of solution as gas microbubbles which attach to or encapsulate the microparticle.

5. The surface stabilized microbubble of claim 1 produced by a method comprising introducing the microparticle having a hydrophobic surface into a liquid which contains gas microbubbles produced by mechanical or chemical means so that

the gas microbubble can attach to or encapsulate the microparticle.

6. A method of enhancing ultrasonic detection in a patient comprising intravenously administering to a patient the surface stabilized microparticle of claim 1 and performing an ultrasound scan on the patient.

7. The surface stabilized microparticle of claim 1 further comprising a drug within the surface stabilized microbubble.

8. A method of delivering a drug to a selected site in a patient comprising

(a) administering to the patient the surface stabilized microbubble of claim 7; and

(b) insonating the selected site in the patient so that the surface stabilized microbubble vibrates or ruptures thereby releasing the drug to the selected target site.

9. The surface stabilized microparticle of claim 1 further comprising a targeting moiety attached to the surface stabilized microbubble.

10. An ecogenic surface comprising an object coated with a hydrophobic surface or a surface with an affinity for a specific gas and gas bubbles which attached to or encapsulate the object.

11. The ecogenic surface of claim 10 produced by a method comprising:

(a) storing the object in a gaseous environment; and

(b) introducing the object into a liquid so that the object carries with it some gas in which it was stored into the

liquid so that gas microbubbles attach to or encapsulate the object.

12. The ecogenic surface of claim 10 produced by a method comprising:

(a) storing the object with an affinity toward a specific gas in the specific gas; and

(b) introducing the object into a liquid so that the object carries with it some gas in which it was stored into the liquid so that gas microbubbles attach to or encapsulate the object.

13. The ecogenic surface of claim 10 produced by a method comprising introducing the object having a hydrophobic surface into a liquid which contains a dissolved gas thereby creating a surface for the dissolved gas to come out of solution as gas microbubbles which attach to or encapsulate the object.

14. The ecogenic surface of claim 10 produced by a method comprising introducing the object having a hydrophobic surface into a liquid which contains gas microbubbles produced by mechanical or chemical means so that the gas microbubbles can attach to or encapsulate the object.

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(71) Applicant *(for all designated States except US)*: **DREXEL UNIVERSITY** [US/US]; 32nd & Chestnut Streets, Philadelphia, PA 19104 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **BASUDE, Raghuveer** [IN/US]; 3604 Spring Garden Street, Apartment D6, Philadelphia, PA 19104 (US). **WHEATLEY, Margaret, A.** [US/US]; 7 Camby Chase, Media, PA 19063 (US).

(74) Agents: **LICATA, Jane, Massey** et al.; Law Offices of Jane Massey Licata, 66 E. Main Street, Marlton, NJ 08053 (US).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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(54) Title: SURFACE STABILIZED MICROBUBBLES FOR USE IN ULTRASOUND CONTRAST AND DRUG DELIVERY AGENTS

(57) Abstract: Surface stabilized microbubbles produced from microparticles or objects having a hydrophobic surface and gas bubbles which attach to or encapsulate the microparticle or surface of the object for use as ultrasound contrast and drug delivery agents are provided.

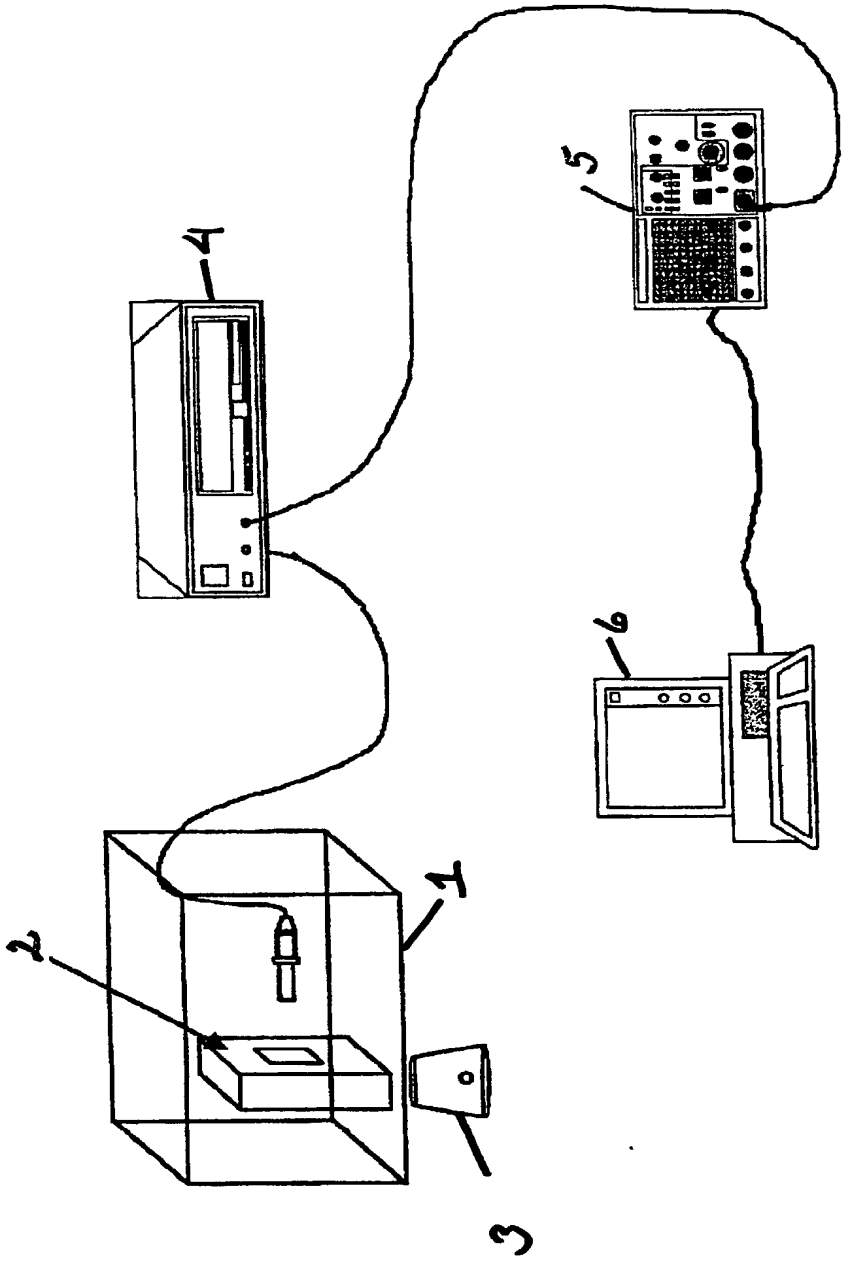

Fig 1.

Docket No.
DRE-0063

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Surface Stabilized Microbubbles for Use in Ultrasound Contrast and Drug Delivery Agents

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on November 29, 2001 as United States Application No. or PCT International Application Number 09/980,134

and was amended on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

60/136,965	June 1, 1999
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

PCT/US00/15161	June 1, 2000	Pending
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

Send Correspondence to:

Direct Telephone Calls to: *(name and telephone number)*
Jane Massey Licata or Kathleen A. Tyrrell - (856) 810-1515

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Full name of sole or first inventor **Raghuveer Basude**	Raghuveer Basude 06/25/02 Date
Sole or first inventor's signature	Raghuveer Basude
Residence ~~**Philadelphia, Pennsylvania**~~	SOMERVILLE N.J. NJ 08
Citizenship **India**	
Post Office Address ~~**3604 Spring Garden Street, Apt. D6**~~	40 BROOKSIDE AVE, APT 1A RB
~~**Philadelphia, Pennsylvania 19104**~~	SOMERVILLE, NJ. - 08876 RB

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Full name of second inventor, if any **Margaret A. Wheatley**	
Second inventor's signature	M. A. Wheatley Date April 23 '02
Residence **Media, Pennsylvania** PA	
Citizenship **US**	
Post Office Address **7 Canby Chase**	
Media, Pennsylvania 19063	